University of Nevada, Reno

Loss of G protein *gpa-12* activity disrupts axon growth in *C. elegans*

A thesis submitted in partial fulfillment
of the requirements for the degree of

Bachelor of Science in Biology and the Honors Program

by

Young H. Lee

Dr. Scott G. Clark, Thesis Advisor

May, 2015

UNIVERSITY OF NEVADA RENO

THE HONORS PROGRAM

We recommend that the thesis
prepared under our supervision by

Young H. Lee

entitled

Loss of G protein *gpa-12* activity disrupts axon growth in *C. elegans*

be accepted in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE, BIOLOGY

_____

Dr. Scott Clark, Ph.D., Thesis Advisor

_____

Tamara Valentine, Ph. D., Director, **Honors Program**

Abstract

Development of the nervous system requires the coordinated action of several signaling pathway networks. Various signaling pathways, including the highly conserved Wnt signaling pathway and G protein signaling pathways, regulate processes such as cell polarity, motility, and axis formation. In some systems, G proteins have been shown to act in Wnt signaling. However, in *C. elegans*, it is unknown whether G proteins are needed for Wnt signaling. Wnt signaling affects several aspects of *C. elegans* development, including axon growth and neuronal polarity of the ALM and PLM neurons. I used the *mec-4::gfp* reporter, which expresses GFP in ALM and PLM, to investigate whether the G protein α-subunit, GPA-12, is needed for ALM or PLM development . I found that a deletion of GPA-12 causes the anterior PLM process to be truncated. Mutation of the Wnt receptor MOM-5, or Wnt signal transducer DSH-2, causes a similar shortening of the PLM process, suggesting that MOM-5, DSH-2 and GPA-12 might act in the same Wnt signaling pathway. I found that 48% of the PLM processes stop prior to the vulva in young, adult *gpa-12* mutants, whereas only 14% stop before the vulva in wild-type. PLM is born, and extends its process during embryogenesis, which continues to grow during the larval stages as the animal elongates. I found that the extension of the PLM process is similar in *gpa-12* and wild-type L1 larvae, indicating that the growth defect of PLM in *gpa-12* animals occurs during larval stages when PLM must grow as the animal elongates. Although the PLM process is short when MOM-5 and DSH-2 activity is reduced, it is unknown whether the defect is embryonic or post-embryonic. Further experimentation is needed to determine if GPA-12 acts with MOM-5 and DSH-2. Ultimately, the confirmation of the necessity of G-proteins

in Wnt signaling could provide useful information for medicinal applications in the treatment of neurological diseases.

Table of Contents

Abstract……i

Table of Contents……iii

List of Tables……..….iv

List of Figures……...……..….v

Introduction……..1

Methods……2

Results……..4

Discussion……...6

References……..13

List of Tables

Table 1.……12

List of Figures

Figure 1……..…8

Figure 2……..…9

Figure 3…….…...10

Figure 4……11

Figure 5……12

Introduction

Neurological conditions and disorders are one of the leading causes of permanent debilitation in the US. The development of new drugs and therapies requires an understanding of the intricacies of the nervous system. During development, neurons project axons and form synapses with their targets[1]. Several guidance factors act to guide growth cones, including Wnts that signal via Frizzleds, and overactivation or underactivation of this pathway could lead to neurodegenerative disorders[2]. Wnt signaling is a conserved pathway that stimulates various intra-cellular signal transduction cascades, which includes the Wnt/β-catenin dependent pathway[3]. Additionally, Wnt proteins regulate multiple cellular processes including cell fate, polarity, motility, axis formation, and organogenesis[3]. Frizzleds belong to the G protein coupled receptor gene family, yet the role of G proteins in Wnt signaling has been controversial. However, recent studies in Drosophila and mammalian cells show that G proteins do act in Wnt-Frizzled signaling[4]. These heterotrimeric G proteins are made of up three, different subunits that are associated with the inner surface of the plasma membrane of cells (Gα, Gβ, Gγ) and the transmembrane receptors of hormones. When a ligand binds to a specific G protein-coupled receptor (GPCR), an allosteric change takes place in the receptors that causes GDP to be replaced by GTP, which facilitates Gα activation and dissociation from Gβ and Gγ, ultimately allowing Gα to activate an effector molecule[5]. In certain Wnt-Frizzled signaling pathways, G proteins are activated to perform specific functions. For example, Wnt-5A is expressed in mouse astrocytes, which induces the recruitment of $G\alpha_{i/o}$ in microglia[4].

C. elegans has 20 Gα, 2 Gβ, and 2 Gγ genes, with one homologue for each of the four mammalian classes of Gα genes, which include: $G\alpha_{i/o}$, $G\alpha_s$, $G\alpha_q$, and $G\alpha_{12}$[6]. *gpa-12*, which is an ortholog included in the subfamily $G\alpha_{12}$, is limited in terms of expression in the pharynx, hypodermis, and ALM/PLM neurons. In *C. elegans*, Wnt signaling can dictate the development of mechanosensory neurons AVM, PVM, ALM, and PLM to varying degrees[7]. Wnt signaling can also regulate the growth and migration of these processes[7].

Loss of *gpa-12* causes a distinct, subtle phenotype. Specifically, animals with the *gpa-12* induced *pk322* deletion (Figure 1), which removes the entirety of the *gpa-12* 3' end, displays sluggish behavior. I used the *mec-4::gfp* reporter to visualize ALM and PLM and found that the anterior PLM process is shortened. I have confirmed the genotypes of the mutant and wild-type strains via PCR and gel electrophoresis. Further study is necessary in order to support the relationship between G proteins and Wnt signaling, which can include conducting an RNAi experiment, as well as developing crosses with other mutant strains.

Methods

The strains used in the study were: *mec-4::gfp(zdIs5),* N2 wild-type, *gpa-12(pk322).* The worms were raised at 20°C under the conditions as outlined by Brenner[9]. The worms were handled via picking methods described by Stiernagle[10].

To observe ALM and PLM, a *mec-4::gfp, gpa-12* double was generated. N2 males were crossed into *mec-4::gfp(zdIs5)* and then gfp/+ males were crossed into *gpa-12*

hermaphrodites and gfp/+;*gpa-12*/+ hermaphrodites were obtained. Twelve L4 heterozygous, hermaphroditic animals, which were screened by presence of GFP on a LEICA MZFLIII microscope, were picked onto separate plates to self-fertilize. At the L4 stage, the F2 progeny were then screened for the presence of both GFP and the *unc* behavioral phenotype. Of the twelve plates, four were selected for homozygosity in the *gpa-12* mutation and GFP by scanning for the presence of a shortened PLM on a LEICA DMRE microscope under 400x magnification, which is shown in Figure 2B.

To calculate penetrance of a shortened PLM, as shown in Figure 3, *gpa-12;mec-4::gfp* and *mec-4::gfp* animals were scored via LEICA DMRE microscope under 400x magnification as young adults (24 hours after L4), which is displayed in Figure 2A and Figure 2B. A shorter PLM was scored when it did not extend beyond the vulva. Additionally, L1 *mec-4::gfp* and L1 *gpa-12;mec-4::gfp* cross animals were scored to determine if the relative shortness was attributed to either an early tiling effect in reaction to ALM, or an outgrowth defect[11]. The criteria used to score the L1 animals was PLM overlap across ALM.

Four, separate 30µl lysates were produced with *zdIs5*, *N2*, *NLS54*, and *NLS54;zdIs5*, using a lysis buffer made up of: 60 ng/µl proteinase K, 0.45% Tween 20, 0.45% NP40, and 1X PCR Buffer. Six polymerase chain reactions were conducted with the primers (Eurofins Genomics) in Table 1 using Rint as a positive control for the deletion. The PCR reactions were made up of: 1X PCR buffer, 200nM forward primer, 200nM reverse primer, 200nM DNTPs, DNA lysate, 0.4 units Taq Polymerase. The reactions were run under the following conditions: 95°C for 3 minutes, 95°C for 30

seconds, annealing at 55°C for 30 seconds, 72°C for 2 minutes 36 seconds, 95°C for 30 seconds, repeating for 39 cycles, and extension at 72°C for 5 minutes. These PCRs were then run on a 0.8% agarose gel alongside a 1kb ladder, as shown in Figure 3, for genotype confirmation.

Results

mec-4::gfp;gpa-12 mutants were generated using standard methods (see Methods). In order to confirm the genotypes, I used PCR and gel electrophoresis (Figure 3). Lanes 5 and 7 display bands at 440 bp, and lane 8 displays a band at 2.5kb. The lane that does not contain mutant lysate (Lane 7), should display bands at 2.5kb and 440bp. The other lane lacking mutant lysate, Lane 3, should not display a band. In the lanes that are positive with the mutant lysate, Lanes 4 and 5 should display a band at 2.5kb and 440kb, whereas Lanes 8 and 9 should only display a 2.5kb band, which are all consistent with the *gpa-12:pk322* deletion.

In addition to developing a mutant cross for further experimentation, young adult *mec-4::gfp* and *gpa-12* animals were scored in order to determine the penetrance of the truncated PLM. Figure 4 shows that in *gpa-12* mutants, the penetrance for the target phenotype in young adults is 48%, compared to 14% for wild-type. Low penetrance for the truncated PLM phenotype, in a wild-type strain, is normal relative to the *zdIs5* phenotype. Additionally, L1 animals were scored in order to determine if PLM shortness is affected in the earlier stages of development (Figure 5). 77% of animals displayed a

shorter PLM in *gpa-12* mutants, compared to 80% for wild-type, which suggests that PLM truncation is due either to an outgrowth defect, or a tiling defect.

Discussion

The lanes that displayed bands at 321bp and 440bp (Lanes 5 and 7), with the Rev2 and Rint primer, respectively, are consistent with what is expected for both the mutant cross and wild-type(N2); the wild-type strain should display a band at 440bp with Rint, indicating a lack of deletion, as well as a band at 2.5kb. Rev2, with the mutant lysate, should display a band at 321bp, which also confirms the presence of the mutation. Furthermore in the mutant, *gpa-12(NLS54)* and *gpa-12;mec-4::gfp*, the gel should not display a band at 2.5kb, and lack a band at 440bp for Rint, which would be consistent with the *pk322* deletion in *gpa-12.* Conversely, in the mutant *gpa-12(NLS54)* and *gpa-12;mec-4::gfp,* the gel should display a band at 321bp for Rev2, which is also consistent with the deletion.

The penetrance values, in the young adults, for the shortened PLM phenotype suggested that the *gpa-12* mutation facilitates truncation of the PLM. After scoring the penetrance of relative PLM length in L1 animals, we found that the penetrance values for both wild-type and the *gpa-12* mutant were nearly the same. Additionally, according to Gallegos and Bargmann, in L1 wildtype animals, the frequency of ALM and PLM overlap is 1.9%[11]. These observations may attribute the mutational phenotype to a tiling or outgrowth defect, both of which are unknown without further study. This could include laser killing various cells via laser ablation, which could help determine how these neurons interact during development[12]. Ultimately, the further investigation of *gpa-12* in Wnt signaling, and its effects on PLM outgrowth, require extensive

experimentation, including RNAi experiments, genetic mapping, and additional mutant crossing.

The study of G proteins has the potential to provide new medicinal approaches to treating neurodegenerative disease. G protein coupled receptors (GPCRs) are widely expressed and involved in a large number of important physiological responses and are relevant in the pharmacological treatment of neurological diseases. Essentially, a better understanding of these complexes can facilitate the advancement of treatments for widespread neurological diseases such as Alzheimer's and Parkinson's disease.

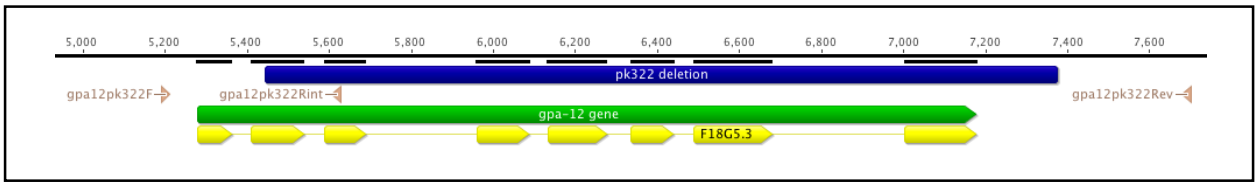

Figure 1. Visual representation of the bp length and position of the *gpa-12pk322* deletion, as well as the position and length of the primers.

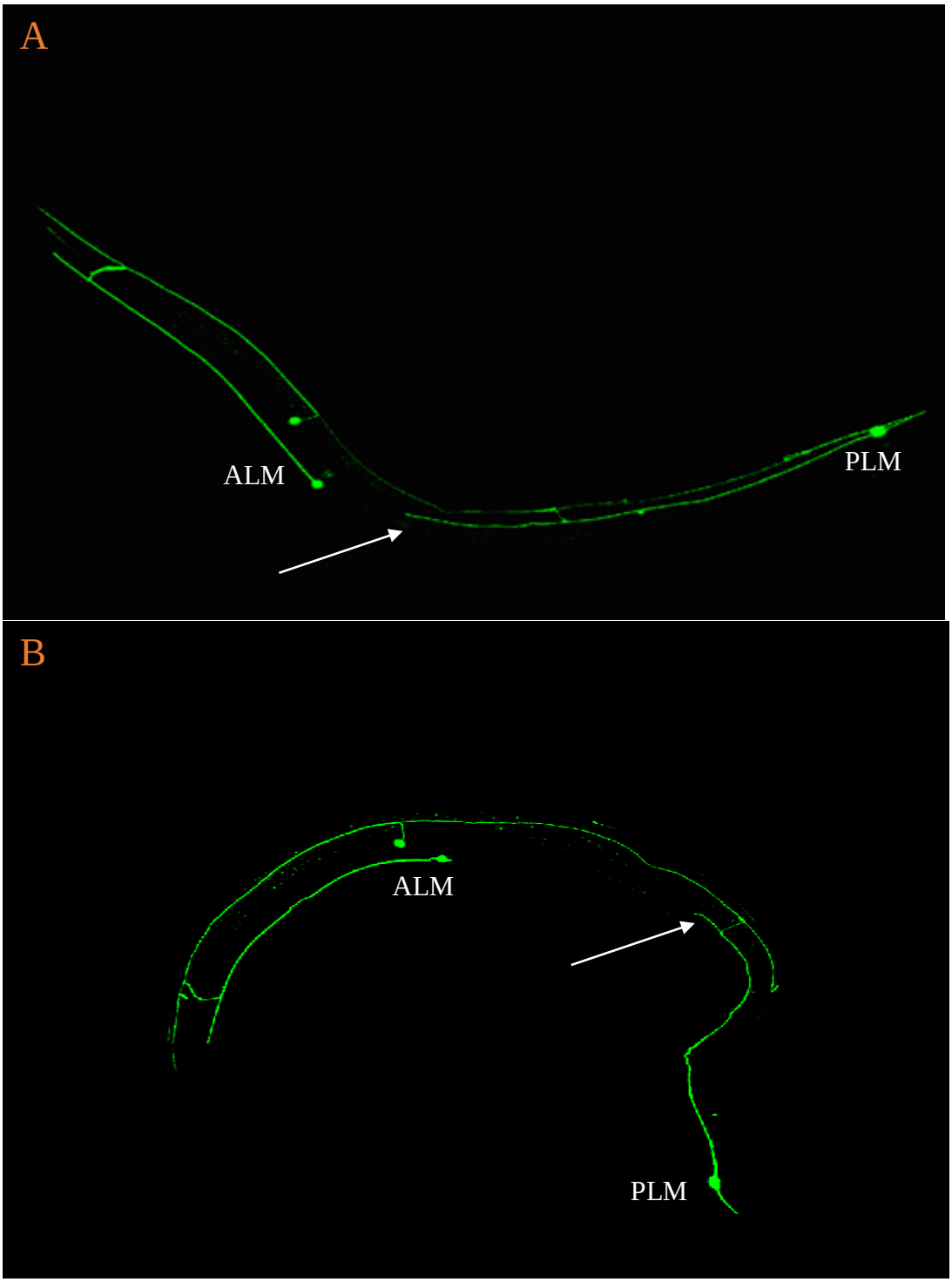

Figure 2. (A) Wild type *zdIs5* hermaphrodite phenotype displaying PLM past the vulva, close to ALM, as indicated by the arrow. (B) *gpa-12* mutant hermaphrodite phenotype displaying a shortened PLM that does not extend past the vulva, as shown by the arrow. This mutation is responsible for the disruption in axon growth.

1 2 3 4 5 6 7 8 9 10

Lane	Primer	Lysate
1	1kb ladder	
2	Empty	
3	F/Rev2	N2
4	F/Rev2	*gpa-12*
5	F/Rev2	*gpa-12;zdIs5*
6	Empty	
7	F/Rint	N2
8	F/Rint	*gpa-12*
9	F/Rint	*gpa-12;zdIs5*
10	Empty	

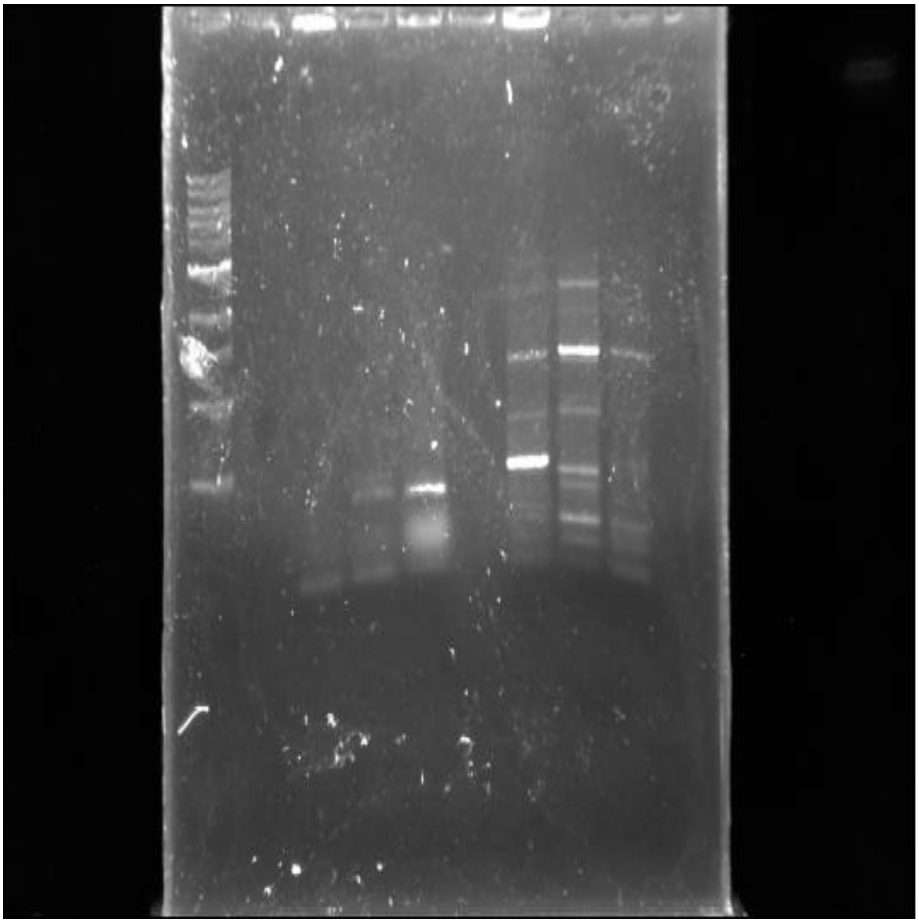

Figure 3. Gel electrophoresis run using a 1kb ladder, N2 lysate, *gpa-12* lysate, mutant cross lysate. Each lysate is run with both forward/reverse 2 primer and forward/reverse internal primer.

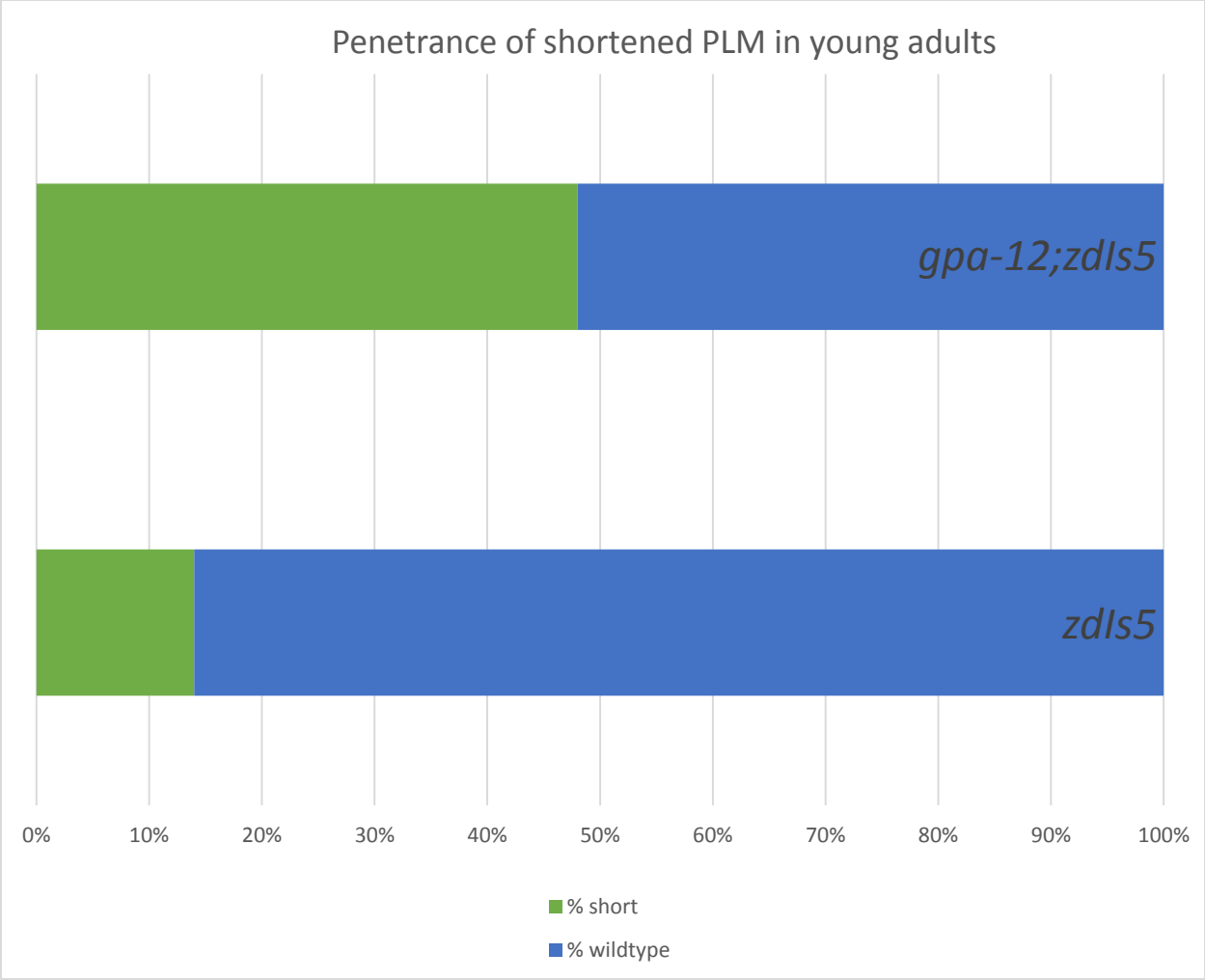

Figure 4. Graph displaying the penetrance frequency of a shortened PLM in both *gpa-12;zd*Is5 and *zdIs5* young adults (24 hours after L4). For *gpa-12;zdIs5*, 48% of young adults presented the shortened PLM phenotype. In *zdIs5*, 14% of young adults exhibited the shortened phenotype.

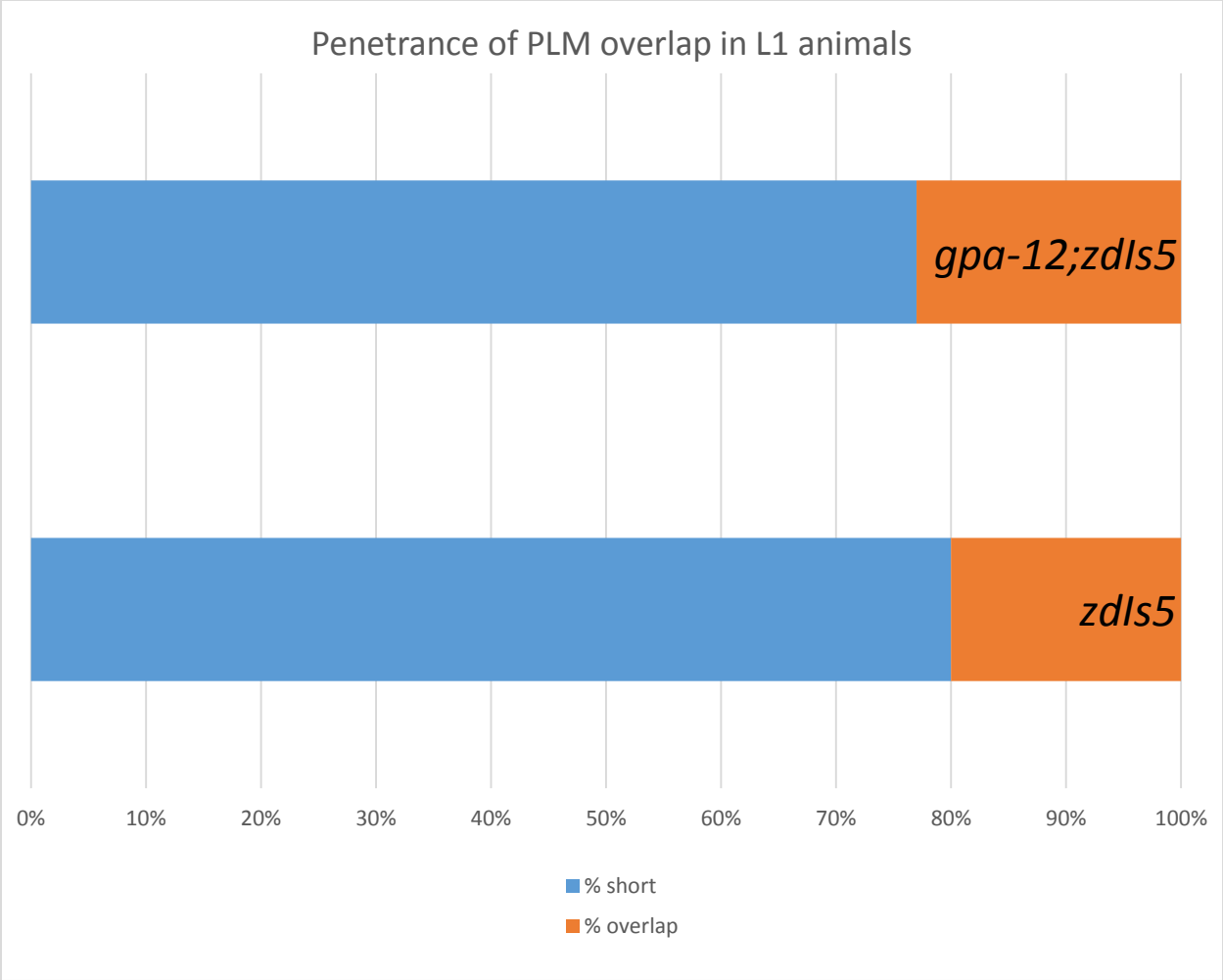

Figure 5. Graph displaying the penetrance frequency of PLM overlap to ALM in both *gpa-12;zdIs5* and *zdIs5* L1 animals. For *gpa-12;zdIs5*, 77% of L1 animals displayed PLMs that did not overlap ALM. In *zdIs5*, 80% of L1 animals presented non-overlapping PLMs.

Table 1. Forward, reverse, and reverse-internal primers used for genotype confirmation of the mutant *gpa-12* cross.

Primers	**Sequence (5' to 3')**	**Amplicon (bp)**
gpa12pk322F	TGACAACAATTGACGCCTGTCTTCC	N/A
gpa12pk322Rint	TGTTCCTAGCATCTAACAACACCCG	440
gpa12pk322R	AGAGTAGATTGGTGCAATTCTGTC	2,300

References

1. Zallen, J.A., Kirch, S.A., Bargmann, C.I. (1999). Genes required for axon pathfinding and extension in the *C. elegans* nerve ring. *Development*, 126(16):3679-3692. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10409513

2. Koval, A., Purvanov, V., Egger-Adam, D., Katanaev, V.L. (2011). Ellow submarine of the Wnt/Frizzled signaling: Submerging from the G protein harbor to the targets. *Biological Pharmacology*, 82:1311-1319.

3. Komiya, Y., Habas, R. (2008). Wnt signal transduction pathways. *Organogenesis*, 4(2):68-75. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2634250/

4. Halleskog, C., Dijksterhuis, J.P., Kilander, M.B.C., Becerril-Ortega, J., Villaescusa, J.C., Lindgren, E., Arenas, E., Schulte, G. (2012). Heterotrimeric G protein-dependent WNT-5A signaling to ERK1/2 mediates distinct aspects of microglia proinflammatory transformation. *Journal of Neuroinflammation*, 9:111. Retrieved from http://www.jneuroinflammation.com/content/9/1/111

5. Purves, D., Augustine, G.J., Fitzpatrick, D. (2001). G-Proteins and Their Molecular Targets. *Neuroscience 2nd edition*. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK10832/

6. Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., Plasterk, R.H.A. (1999). The complete family of genes encoding G proteins of Caenorhabditis elgans. *Nature*, 21:414-419.

7. Prasad, B.C., Clark, S.G. (2006). Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in C. elegans. *Development*, 133(9):1757-1766. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16571624

8. Park, E., Horvitz, H.R. (1986). *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle. *The Genetics Society of America*, 113:853-867. Retrieved from http://www.genetics.org/content/113/4/853.full.pdf

9. Brenner, S. (1974). The Genetics of CAENORHABDITIS ELEGANS. *Genetics*, 77(1):71-94. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1213120/

10. Stiernagle, T. (2006). Maintenance of *C. elegans*. *WormBook*. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK19649/#A7966

11. Gallegos, M.E., Bargmann, C.I. (2004). Mechanosensory Neurite Termination and tiling Depend on SAX-2 and the SAX-1 Kinase. *Neuron*, 44:239-249.

12. Bargmann, C.I., Avery, L. (1995). Laser Killing of Cells in *Caenorhabdtis elegans*. *Methods in Cell Biology*m, 48:225-250. Retrieved from http://elegans.som.vcu.edu/~leon/cv/papers/laser_methods/paper.pdf